served with IL-1β+Ro treatment. Furthermore, western blot studies indicated that IL-1β+Ro did not induce the time-dependent activation of caspase-7 and -3 observed with TNF-α+Ro. The antiapoptotic protein bcl-2 expression has not been altered in a different manner by TNF-α+Ro or IL-1β+Ro. In order to elucidate the role of the caspases on the effect of TNF-α on synoviocytes cell death induced by Ro, caspase inhibitors (-3, -3/7, -8 and general) effects were analized by cell death and activation of the executioner caspase-3 studies. Results show that preincubation for two hours with all inhibitors significantly decreased the percentage of apoptotic cells by treatment with TNF-α+Ro at 24 hours (1,88±0,29%, 3,76±2,04%, 2,28±0,77%, and 2,8±1,59% with caspase-3, caspase-3/7, caspase-8 and caspase general inhibitor, respectively, n=2, p<0,0001). When caspase-3 activation was evaluated by western blot, it was found that preincubation of caspase-8 inhibitor was effective to prevent caspase-3 induced by TNF-α+Ro. By contrast, caspase-8 inhibitor not prevented bcl-2 decrease induced by TNF-α+Ro or IL-1β+Ro.
Conclusions: These results confirm that the cytokines TNF-α and IL-1β differently regulate machinery apoptotic activation in synoviocytes. In addition, this difference is dependent on caspase-8 expression levels. These data could be important for a better understanding of the participation of TNF-α and IL-1β in the OA pathogenesis.

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SCA-1 POSITIVE CELLS FROM THE SYNOVIUM OF AN ADULT JAPANESE WHITE RABBIT CAN DIFFERENTIATE INTO NEURON-LIKE CELLS

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Purpose: Bari *et al.* showed that fibroblast-like cells from synovial tissue could differentiate into osteoblasts, chondrocytes, adipocytes, and muscle cells. However, there are no reports showing the differentiation ability of stem cells from the synovium. In this study, we detected Sca-1 positive cells from synovium of adult Japanese white rabbit, and tried to differentiate into neuron-like cells.
Methods: 1. Presence of Sca-1 positive cells;
To demonstrate the presence of Sca-1 positive cells, we performed the immunohistological analysis of the synovium using Sca-1 antibody.
2. Differentiation into neuron-like cells with Sca-1 positive cells and culture of Sca-1 positive cells;
We isolated and cultured the synovium cells and performed FACS analysis with Sca-1 antibody. To evaluate the differentiation ability of Sca-1 positive cells into neuron-like cells, we cultured the Sca-1 positive cells using the nerve progenitor cell maintenance medium, and performed morphological and biochemical analysis.
Results: 1. Presence of Sca-1 positive cells;
In the immunohistological staining, Sca-1 positive cells were observed in the synovium of adult Japanese white rabbit.
2. Differentiation into neuron-like cells with Sca-1 positive cells and culture of Sca-1 positive cells;
The result of the FACS analysis showed that the average 3.99±1.20% of total cultured cells form synovium was Sca-1 positive cells.
Morphological analysis showed neurosphere formation in the culture. Biochemical analysis also showed that nestin- positive cells were observed in the neurosphere, suggesting that Sca-1 positive cells could differentiate into nestin-positive cells.
Moreover, we cultured nestin-positive cells using the medium with b-FGF and serum, and performed the morphological and biochemical analysis. In this culture, a successful differentiation into neurofilament-M-positive cells were apparently achievable, suggesting that Sca-1 positive cells from synovium differentiated into neuron-like cells via nestin-positive cells.
Conclusions: Sca-1 positive cells from synovium of adult rabbit could differentiate into neuron-like cells.

Tissue Engineering and Repair

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BIOFUNCTIONAL POLY(PROPYLENE SULFIDE) NANOPARTICLES FOR TARGETED DRUG DELIVERY: IDENTIFICATION OF A NOVEL TARGETING PEPTIDE FOR ARTICULAR CARTILAGE BY PHAGE DISPLAY

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Purpose: Increasing knowledge of molecular mechanisms in the pathogenesis of osteoarthritis leads to the identification of possible drug targets and drug development. As systemic drug administration is associated with a low bioavailability in the cartilage matrix and direct injection results in a fast clearance out of the joint, nanoparticle-based localized intra-articular drug delivery with an active targeting mechanism may enhance the compound's bioavailability in articular cartilage and thus the treatment efficacy while reducing adverse systemic effects. This may particularly be favorable in clinical entities with early cartilage degradation, such as femoro-acetabular impingement in the hip.
Methods: In order to surface-functionalize poly(propylene sulfide) (PPS) nanoparticles, a phage display library has been screened against bovine cartilage grafts to find a novel targeting peptide for the cartilage matrix. Five cycles of biopanning have been carried out with increasing stringency of binding conditions in each cycle. The sequence of the displayed peptide was obtained by DNA sequencing after cycle 5 of retained and amplified phage clones. Binding assays have been carried out in vitro against bovine cartilage in physiological conditions (37°C, with and without synovial fluid). The selected peptide and its mismatch were synthesized using standard Fmoc-chemistry with a cysteine at the C-terminus. The targeting peptide was then conjugated via the thiol group of the cysteine by Michael-type addition to Pluronic F-127 which was previously functionalized with vinylsulfone. PPS nanoparticles were prepared in an inverse emulsion polymerization with conjugated (10%) and non-conjugated (90%) Pluronic F-127 as the emulsifier, such that an average size of 38nm was obtained. After polymerization of the PPS core, the Pluronic with the conjugated targeting peptide remains displayed on the particle surface, thereby forming a surface-functionalized nanoparticle.
Results: DNA sequencing revealed three putative peptide sequences after 5 cycles. All of these sequences have been shown to be specific to cartilage versus synovial membrane by two orders of magnitude. A competetive binding assay between the three phage clones and the original library retained only two phage clones. The free peptide of the clone with the highest titer per ml was subjected to a competetive binding assay against the corresponding phage clone, which resulted in an IC50 of the free peptide of 200nM, suggesting a fairly high affinity to its target. Similarly, the surface-functionalized nanoparticle was subjected to a competetive binding assay against the corresponding phage clone and compared to the free peptide and its mismatch. The conjugated nanoparticles at a concentration of 2.5% w/v exhibited similar binding as the free peptide at a concentration of 10M (10.4Â±6% vs.13.9Â±2.8% of control), whereas the mismatch peptide did not bind competetively and thus did not reduce the phage titer (92Â±12%).

Conclusions: In summary, we have characterized a novel targeting peptide and showed its specificity as well as its competetive binding to articular cartilage. Surface functionalisation of PPS nanoparticles was carried out and showed similar binding at 10% surface functionalisation as the free peptide. After further optimisation, the functionalized nanoparticles will be labeled and loaded with small molecule drugs to determine the in vivo targeting of the articular cartilage and their capability of sustained intra-articular drug release in the cartilage matrix.

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MECHANO-ACTIVE CARTILAGE TISSUE ENGINEERING USING A HIGHLY ELASTIC SCAFFOLD AND BONE MARROW STEM CELLS

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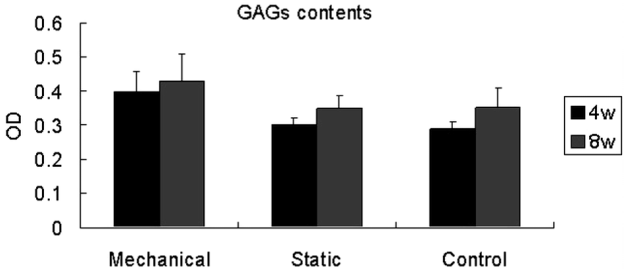
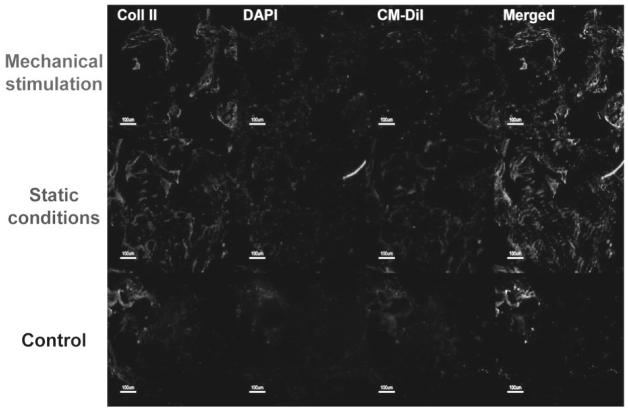
Purpose: Articular cartilage is subjected to complex loading, which include compressive and shearing forces. These mechanical forces play a major role in the growth, development and maintenance of the articular cartilage in the body. It is known that proper stimulation is necessary to promote chondrogenesis. This means the compressive mechanical stimulation is a very important factor for the formation of articular cartilage using a tissue engineering technique. Therefore, it is essential to develop a mechano-active bioreactor that can deliver the mechanical signals to adherent cells on polymer scaffolds during the application of mechanical strain application. The objective of this study is to evaluate the effect of dynamic compression for the differentiation of bone marrow stromal cells (BMSCs) within an elastic scaffold and the formation of cartilaginous tissue.
Methods: The mechano-active scaffolds were fabricated from a very elastic poly(L-lactide-co-caprolactone) (PLCL) with 85% porosity and a 300∼500 μm pore size using a gel-pressing method. The scaffolds were seeded with BMSCs and the continuous compressive stimulation was applied at 0.1Hz for 10 days in chondrogenic media in order to evaluate the effect of dynamic compression on differentiation of BMSCs and the secretion of the chondral extracellular matrix. In addition, the BMSCs seeded constructs were implanted subcutaneously into nude mice to determine their biocompatibility and cartilaginous tissue formation. Cell-polymer constructs were characterized by biochemical analysis, histological studies, and immunofluoresecence staining. For defining the gene expression for mechanical stimulation, reverse transcription-polymerase chain reaction was performed.
Results: Mechano-active scaffolds having a complete rubber-like elasticity were prepared by a gel-pressing method. They could be easily twisted and bended and showed almost complete (over 97%) recovery at strain applied of up to 500%. In *in vitro* tests, the accumulation of extracellular matrix of cell-polymer constructs showed that chondrogenic differentiation was sustained and enhanced significantly by dynamic compressive stimulation. The GAGs contents of implants stimulated by the dynamic compressive deformation were higher than them without stimulation. Histological analysis showed that implants stimulated mechanically by compression formed mature and well-developed cartilaginous tissue, as evidenced by chondrocytes within lacunae and an abundant accumulation of sulfated GAGs. From the results, the periodic application of dynamic compression can encourage bone marrow stromal cells to differentiation to chongenic lineage and to maintain their phenotypes. Consequently, it may improve the quality of cartilaginous tissue formed in vitro and in vivo.





Conclusions: In conclusion, the appropriate periodic application of dynamic compression can encourage the BMSCs to maintain their phenotypes, promote differentiation and enhance GAGs and type II collagen production, as evidenced by the improved quality of the cartilaginous tissue formed *in vitro* and *in vivo*. Therefore, it is believed that tissue engineering techniques using mechao-active PLCL scaffolds and dynamic compression would be very beneficial for cartilaginous tissue formation.

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ASSESSMENT OF CARTILAGE REPAIR TISSUE, FOLLOWING AUTOLOGOUS CHONDROCYTE IMPLANTATION, BY HISTOCHEMISTRY AND FOURIER TRANSFORM INFRARED IMAGING SPECTROSCOPY

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Purpose: Current methods used to repair localized articular cartilage defects include autologous chondrocyte implantation (ACI). There has been limited success in correlating clinical outcome with quality of the repair tissue formed. More sophisticated techniques that can assess the molecular features of repair tissue would aid the evaluation of treatments. Fourier transform infrared imaging spectroscopy (FT-IRIS) can be used to assess articular cartilage based on molecular vibrations that arise from its primary constituents, collagen and proteoglycan (PG). In this study tissue biopsies obtained one year post-ACI treatments were analyzed by immunohistochemistry and FT-IRIS to assess the molecular features of the repair tissue.
Methods: Five male patients (aged 21, 25, 34, 42 and 52 yrs) were treated with ACI for cartilage defects. Their follow-up treatment included an arthroscopic assessment and biopsy of the treated area at 12 months post-treatment. Biopsies were sectioned for histological, immunohistochemical (collagen types I and II) and FT-IRIS analyses and compared to normal human articular cartilage. Sections on BaF_2 windows were analyzed